

Soil biodiversity and soil community composition determine ecosystem multifunctionality

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Biodiversity loss has become a global concern as evidence accumulates that it will negatively affect ecosystem services on which society depends. So far, most studies have focused on the ecological consequences of above-ground biodiversity loss; yet a large part of Earth's biodiversity is literally hidden below ground. Whether reductions of biodiversity in soil communities below ground have consequences for the overall performance of an ecosystem remains unresolved. It is important to investigate this in view of recent observations that soil biodiversity is declining and that soil communities are changing upon land use intensification. We established soil communities differing in composition and diversity and tested their impact on eight ecosystem functions in model grassland communities. We show that soil biodiversity loss and simplification of soil community composition impair multiple ecosystem functions, including plant diversity, decomposition, nutrient retention, and nutrient cycling. The average response of all measured ecosystem functions (ecosystem multifunctionality) exhibited a strong positive linear relationship to indicators of soil biodiversity, suggesting that soil community composition is a key factor in regulating ecosystem functioning. Our results indicate that changes in soil communities and the loss of soil biodiversity threaten ecosystem multifunctionality and sustainability.

microbiome | ecology | symbiosis | global change | soil degradation

It has long been recognized that biodiversity can be the mechanism behind the performance of an ecosystem, particularly in communities of above-ground organisms (1–5). In soils below ground, however, the functioning of biodiversity is not well understood (6). Soils are highly diverse. It has been estimated that 1 g of soil contains up to 1 billion bacteria cells consisting of tens of thousands of taxa, up to 200 m fungal hyphae, and a wide range of mites, nematodes, earthworms, and arthropods (7, 8). This vast and hidden diversity contributes to the total terrestrial biomass and is intimately linked to above-ground biodiversity (9, 10).

In recent years several studies have shown that anthropogenic activities, such as agricultural intensification and land use change, reduce microbial and faunal abundance and the overall diversity of soil organisms (11–13). This has triggered increasing concern that reduced biodiversity in soils may impair numerous ecosystem functions, such as nutrient acquisition by plants and the cycling of resources between above- and below-ground communities (6, 11, 13, 14). However, to date research has largely focused on the effects of specific groups of organisms, such as soil microbes (15, 16), mycorrhizal fungi (17, 18), and soil fauna (19, 20), or on large-scale correlative analysis in the field (13). However, soil organisms interact within complex food webs, and therefore changes in diversity within one trophic group or functional guild may alter the abundance, diversity, and functioning of another (21, 22). Hence, it is important to know how changes in soil biodiversity and the simplification of the soil community composition influences ecosystem functioning. However, whether reductions of biodiversity in soil communities have consequences for the overall performance of an ecosystem remains unresolved. Moreover, recent studies show that above-

ground plant diversity influences multiple ecosystem functions, defined as ecosystem multifunctionality (23). However, it is still unclear whether ecosystem multifunctionality is likewise influenced by soil biodiversity.

Here we manipulated soil biodiversity and soil community composition in model grassland microcosms simulating European grassland. We tested whether changes in soil biodiversity and soil community composition influenced multiple ecosystem functions. To manipulate soil biodiversity and soil community composition, we inoculated the grassland microcosms with different soil communities. The soil inoculum was prepared by fractionating soil communities according to size, using filters of decreasing mesh size (19). This method reduces the abundance of different groups of soil organisms at different mesh sizes, thus altering the community composition and the overall diversity of soil organisms simultaneously (19). To maintain the different soil community treatments and to prevent microbial contamination, we maintained the communities in self-contained microcosms in which we could restrict external contamination (24). Additionally, the experiment was repeated and performed for a longer period to confirm initial results and include additional measures on ecosystem characteristics. We hypothesized that soil biodiversity loss reduces ecosystem functioning and multifunctionality. Specifically, we hypothesized that plant diversity, decomposition, and the recycling of nutrients is impaired when the diversity and abundance of various groups of soil biota (e.g., fungi, mycorrhizal fungi, bacteria, and nematodes) are reduced.

Significance

Biological diversity is the foundation for the maintenance of ecosystems. Consequently it is thought that anthropogenic activities that reduce the diversity in ecosystems threaten ecosystem performance. A large proportion of the biodiversity within terrestrial ecosystems is hidden below ground in soils, and the impact of altering its diversity and composition on the performance of ecosystems is still poorly understood. Using a novel experimental system to alter levels of soil biodiversity and community composition, we found that reductions in the abundance and presence of soil organisms results in the decline of multiple ecosystem functions, including plant diversity and nutrient cycling and retention. This suggests that below-ground biodiversity is a key resource for maintaining the functioning of ecosystems.

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Results and Discussion

We successfully obtained a broad soil biodiversity gradient in our grassland microcosms (Fig. 1 and *SI Appendix*, Figs. S1 and S2 and Table S1). Some groups of soil organisms (e.g., nematodes and mycorrhizal fungi) were entirely eliminated within the gradient, whereas fungal and bacterial communities showed reduced abundance and richness (Fig. 1). This resulted in an overall shift in soil community composition and in a decline in the diversity of soil biota in each soil community treatment along our gradient.

Changes in the soil communities across the gradient influenced various ecosystem functions (Fig. 2). Among the ecosystem functions assessed, plant species diversity declined strongly with reductions in soil biodiversity and simplification of the soil communities (Fig. 2), supporting previous reports that plant community composition is driven by the diversity and species composition of various groups of soil organisms (17, 19, 25). Legumes and forbs declined in productivity as soil biodiversity was depleted, whereas grasses increased in productivity in the most simplified soil communities, contributing up to 92% of the net primary productivity (*SI Appendix*, Figs. S3 and S4). Carbon sequestration also declined along the gradient (Fig. 2). However, this effect was relatively small because this function is likely mediated more by a combination of plant and soil community characteristics than a direct function of soil biodiversity alone (26, 27).

The changes in soil biodiversity and soil community composition also influenced processes related to nutrient cycling. Changes in ecosystem processes that retain nutrients within the system are linked to the ability of soil organisms to break down organic matter and recycle liberated resources back into the above-ground community (10). Specifically, the decomposition of plant litter and the reincorporation of the nitrogen liberated from the litter back into above-ground plant tissues declined as overall soil biodiversity was reduced and with simplification of the soil communities (Fig. 2). Moreover, phosphorus loss through leaching after a simulated rain increased exponentially with successive simplification of soil communities, reaching up to a threefold loss in the most simplified soil community (Fig. 2). These results support past observations and hypotheses suggesting

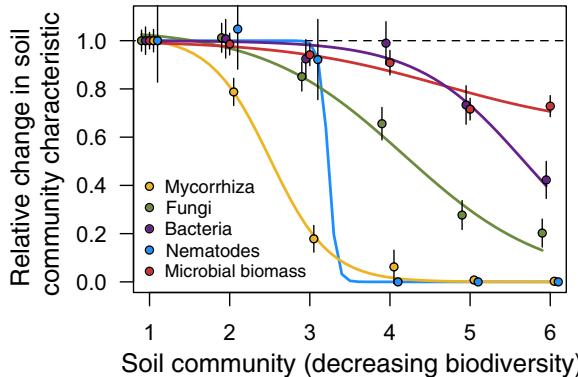


Fig. 1. Change in soil community characteristics in grassland communities with increasing simplification of soil communities, according to size. Soil communities were established by filtering through different meshes: 1 $\leq 5,000 \mu\text{m}$, 2 $\leq 250 \mu\text{m}$, 3 $\leq 50 \mu\text{m}$, 4 $\leq 25 \mu\text{m}$, 5 $\leq 10 \mu\text{m}$, and 6 sterilized soil. These measures reflect both abundance (nematodes, mycorrhizal colonization of plant roots, and microbial biomass) and richness (bacteria and fungal richness) of various guilds of soil organisms. Means \pm SEM are expressed as a ratio of the most complete soil treatment (soil community 1, dashed line), such that 0 represents no detection (raw data in *SI Appendix*, Figs. S1 and S2). Where no error bars are shown for mycorrhiza and nematodes they were not detected in any replicate. Lines highlight the general trend in changes in the soil community characteristics along the gradient. Soil community characteristics measured in both experiments are pooled.

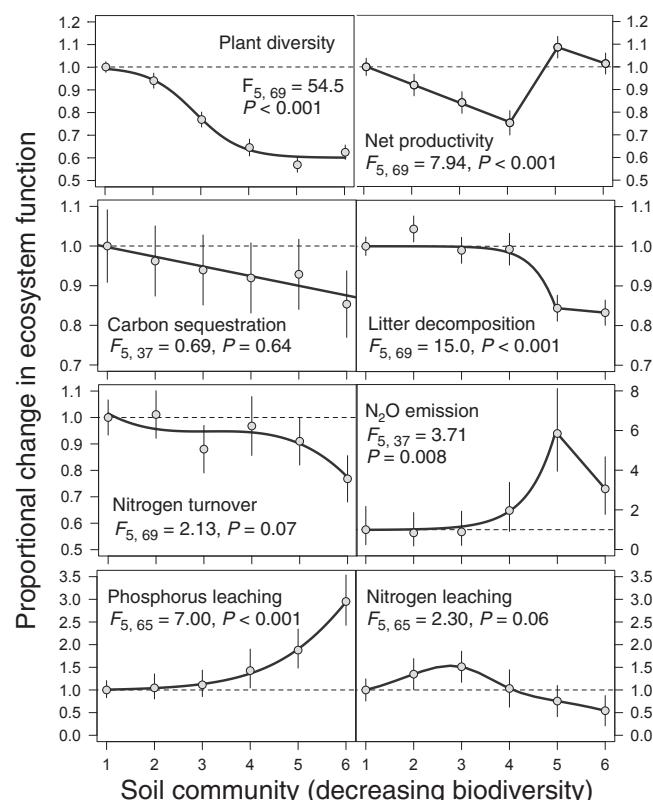


Fig. 2. Change in ecosystem functions in grassland communities along the continuum of increasingly simplified soil biotic communities. Means \pm SEM of plant productivity (g), plant diversity (Shannon index), N turnover (shoot $\delta^{15}\text{N}$), decomposition (%), C sequestration (soil $\delta^{13}\text{C}$), N leaching (mg), P leaching (mg), and N_2O emissions (mg m^{-2}) are expressed as a ratio of the most complete soil treatment (soil community 1, dashed line) such that values below 1 represent a reduction, and values above 1 indicate an increase in the ecosystem function (raw data in *SI Appendix*, Fig. S5). Lines highlight trends in the changes in ecosystem functions across the gradient. Ecosystem functions measured in both experiments are pooled (Results of the individual experiments are given in *SI Appendix*, Figs. S6–S8 and Table S1). Soil communities are based on organism size as described in Fig. 1.

that a greater diversity of soil organisms can enhance litter breakdown, reduce nutrient leaching losses, and maintain resource turnover between above- and below-ground communities (10, 11, 14). The loss of nitrogen via N_2O emissions also increased up to sixfold in the second most simplified soil community (Fig. 2). This demonstrates that the simplification of soil biotic communities alters nitrogen transformation processes in the soil, resulting in increased emission of N_2O , which is an important greenhouse gas (28).

To assess the overall performance of the grassland microcosms, we averaged the standardized scores (z scores) of all ecosystem functions (Fig. 2) to obtain a single index of ecosystem multifunctionality (23). We combined the soil community characteristics (Fig. 1) in the same manner to obtain a single index reflecting the soil biodiversity within the microcosms created by filtering. This biodiversity index therefore reflects the overall community compositional changes in concert with changes in soil biodiversity. Overall, the changes in ecosystem multifunctionality showed a positive relationship to the average of our indicators of soil biodiversity (Fig. 3), indicating that changes in soil biodiversity impact ecosystem multifunctionality. The large proportion of variation in ecosystem multifunctionality explained by the soil biodiversity index indicates that the soil community characteristics measured were appropriate indicators of soil biodiversity in our system. Ecosystem multifunctionality did not

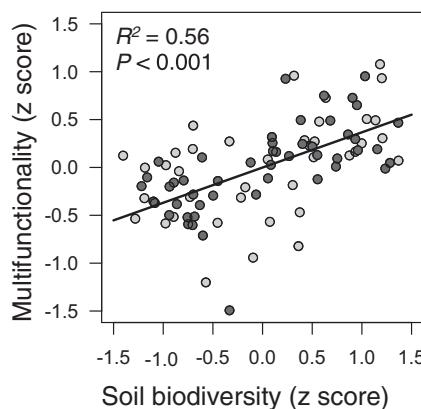


Fig. 3. Ecosystem multifunctionality index in relation to the soil biodiversity index. Lightly shaded points represent grassland communities in experiment 1, and darkly shaded points indicate grassland communities in experiment 2. The overall regression is shown pooled for both trials because there was no difference between trials in the overall relationship between the soil biodiversity (the combined measures shown in Fig. 1) and ecosystem multifunctionality (the combined ecosystem functions shown in Fig. 2). The relationship of individual ecosystem functions to the soil biodiversity score is shown in *SI Appendix*, Fig. S6, and changes in the two indices across the gradient of soil communities are shown in *SI Appendix*, Fig. S7.

vary strongly between the two initial levels of soil community simplification (e.g., soil communities consisting of organisms up to 5,000 μm or 250 μm), and a strong reduction in ecosystem multifunctionality was only observed in highly simplified soil communities (*SI Appendix*, Fig. S7). This supports the theory that at higher levels of soil biodiversity ecosystem functions are robust to changes in soil biodiversity and composition of soil biota (21). Moreover, similar to changes in multifunctionality, our index of soil biodiversity, also did not vary strongly between the two initial levels of soil community simplification. Additionally, as soil communities became increasingly simplified, the loss or strong suppression of key groups of soil organisms (e.g., mycorrhizal fungi and nematodes) corresponded with an abrupt shift in many of the ecosystem functions (Fig. 2 and *SI Appendix*, Fig. S8). This highlights that broad-scale changes in the soil community may be tightly linked to the overall functioning of the ecosystem and that ecosystem functioning is likely more sensitive to changes in the presence and abundance of various soil organisms when overall biodiversity is low (21).

Our results were obtained in two independent experiments, and results of both experiments were similar, pointing to the robustness of our findings. The effects of changes in soil biodiversity and community composition on decomposition of plant litter and nutrient turnover were stronger in the second experiment (*SI Appendix*, Fig. S8), which was the longer-lasting experiment. This suggests that the consequences of simplified soil community composition and reduced soil diversity may become progressively more inhibiting as time passes. Additionally, because plant diversity is also a driver of ecosystem multifunctionality (3–5, 23), the strong effects of soil organisms on plant diversity, observed here and elsewhere (15–20), could indirectly influence a number of other ecosystem functions, such as nutrient availability (23) and C sequestration (26). A path analysis indeed indicated that effects of soil biodiversity and composition on measures of nutrient losses were, in part, indirect and mediated by soil biodiversity-induced changes in plant diversity and productivity (*SI Appendix*, Figs. S9 and S10 and Tables S2 and S3). Further path analyses assessing the direct and indirect associations between the individual soil community characteristics and ecosystem functions indicate that different components of the soil community differentially influence the

various ecosystem functions (*SI Appendix*, Figs. S4 and S5 and Tables S4 and S5).

Two decades of biodiversity research have shown that above-ground plant diversity is a key driver of ecosystem functioning in a wide range of ecosystems (2–5). Our research extends this observation to the below-ground environment, suggesting that a reduction of soil biodiversity and changes in soil community composition impacts not only on the associated plant community but also on a number of key ecosystem processes that are necessary to maintain overall ecosystem performance. These findings are in line with a recent large-scale correlative field study that indicates that soil food web properties are associated with ecosystem services across various European land use systems (13). The predicted suppression of soil biodiversity due to chronic disruptions to soil communities through intensified anthropogenic activities (11–13), coupled with climate change, are likely to negatively influence the performance of multiple ecosystem process (6). Thus, the protection of soil biodiversity is a key issue to be considered in further detail for the sustainability of terrestrial ecosystems.

Materials and Methods

Microcosms, Substrate, Soil, and Plant Communities. Experimental grassland microcosms were established under sterile conditions in closed growth chambers. Incoming air and water entered the microcosms through purifying filters to prevent outside contamination (24) (*SI Appendix*, *SI Materials and Methods*). Microcosms measured 23.5 cm in diameter and had a rooting depth of 12 cm. Each microcosm was filled with 6 kg of a standard sterile soil (96% soil volume) and an inoculated soil community (4% soil volume). Different soil community inoculum treatments were created by sequentially sieving 250 g field soil through a series of decreasing mesh sizes: soil organisms $\leq 5,000 \mu\text{m}$, $\leq 250 \mu\text{m}$, $\leq 50 \mu\text{m}$, $\leq 10 \mu\text{m}$, and sterile soil in experiment 1. In experiment 2 an additional $<25\text{-}\mu\text{m}$ soil community treatment was added to the design. The inoculum fraction not passing through the sieve was sterilized and was mixed throughout the sterile soil in each microcosm along with the fraction passing through the sieve, to ensure that each treatment received the same amount of inoculum. Each soil community treatment was replicated eight times in both experiments, with the exception of 10 replicates of the sterilized soil community in experiment 2, bringing the total experimental units in the two experiments to 40 and 50, respectively. The reduction of soil communities by filtering removes guilds of soil organisms as well as dilutes out their abundance with each additional filter (19, 29).

In each microcosm a plant community consisting of 40 individual plants comprising 10 species that are typical of temperate European grasslands (30) were planted: legumes (five individuals of *Trifolium pratense* and five of *Lotus corniculatus*), grasses (four *Lolium multiflorum*, five *Poa annua*, and five *Festuca pratensis*) and forbs (three *Prunella vulgaris*, two *Senecio jacobaea*, four *Plantago lanceolata*, three *Achillea millefolium*, and four *Capsella bursa-pastoris*). Microcosms were maintained in the greenhouse. The experiment was carried out twice: for a growth period of 14 wk (experiment 1) and for a growth period of 24 wk (experiment 2).

Soil Community Characterization. At the end of each experiment, and after 12 wk for the second trial, soil was removed for molecular and microscopy analyses (details in *SI Appendix*, *SI Materials and Methods*). Bacterial and fungal community composition was determined using ribosomal internal spacer analysis (31–33). Roots were collected and scored for the absence/presence of arbuscular mycorrhizal colonization using an intersect-transect method for 100 intersections (34). The number of nematodes was assessed in a 100-g soil sample (35), and soil DNA was used as an indicator of soil microbial biomass because it corresponds well with other methods that reflect microbial biomass (36, 37).

Ecosystem Functions. At the final harvest, plant shoots were cut at the soil surface, the number of individuals of each species harvested was counted, and shoot biomass was determined. At 12 wk in the second experimental trial, plants were harvested at 5 cm above the soil surface to simulate hay making, typical for many European grasslands, and the number of plants per species harvest was recorded. Net plant productivity was measured as the total plant above-ground biomass. The Shannon-Wiener index of diversity was calculated using the above-ground biomass per individual plant harvested of each species as the surrogate for abundance in the equation.

Litter decomposition was assessed with litterbags containing ^{15}N -labeled sterilized *L. multiflorum* shoots that were added to microcosms at the start of the experiment. The ability for a plant to acquire N through the mineralization of its litter is defined here as "N turnover." N turnover was estimated using the $\delta^{15}\text{N}$ signal in the *L. multiflorum* shoots at the end of the experiment. At the final harvest, microcosms were watered to saturate the soil to roughly 10% beyond the water holding capacity of the soil to induce leaching. Leachate percolating through the soil column was collected from a small outlet at the bottom of the microcosm and was assessed for nutrient concentrations (PO_4 , total P, NO_3 , and NH_4) as described elsewhere (38, 39). Fertilization and water saturation of soil after rainfall events not only facilitate nutrient leaching but also initiate denitrification and the production of N_2O , an important greenhouse gas (40). Hence, N_2O production was measured at the end of the second experiment after fertilizer addition and soil saturation with water. At 48 and 24 h before the final harvest of microcosms in the second experiment 40 mL of $^{13}\text{CO}_2$ (99% ^{13}C) gas was injected into each microcosm, and below-ground ^{13}C allocation was measured. Further details on all measurement procedures are given in *SI Appendix, SI Materials and Methods*.

Soil Biodiversity and Multifunctionality Indices. All soil community and ecosystem function data from each harvest period were standardized by z transformation (overall mean of 0 and SD of 1) and used in all subsequent calculations and analyses. This removed overall differences between trials and harvest time points and simultaneously equalized the variance among measures and sampling time points. Subsequently, the average of all standardized ecosystem functions (*SI Appendix, Fig. S6*) was used as an index of ecosystem multifunctionality following the approach used by Maestre et al. (23). Data for ecosystem functions in which greater values reflect a more undesirable aspect of the ecosystem (increasing nutrient leaching and N_2O production) were multiplied by -1 (inverted around the 0 mean) to maintain directional change with other ecosystem functions, such that a decline from their desirable state corresponds to increasingly negative values. By doing this the general difference among soil community treatments in overall ecosystem functioning could be more easily assessed.

A soil biodiversity index was calculated from the average of all standardized soil community characteristics and used as a general indicator of soil biodiversity and compositional changes. This soil biodiversity index includes measures of richness (bacterial richness, fungal richness) and the relative abundance of guilds of soil organisms (number of nematodes, root colonization by arbuscular mycorrhizal fungi, and an estimate of soil microbial biomass). Thus, the biodiversity index calculated here parallels typical biodiversity indicators by combining measures of richness and relative abundance

(41). Note, however, that more ecosystem functions (carbon sequestration and N_2O emissions) and soil community characteristics (nematode abundance) were measured in the second experiment and are incorporated into the biodiversity and multifunctionality indices of the second experiment even though absent from the first.

Data Analyses. All data on ecosystem functions and soil community characteristics were assessed for variation among soil community treatments in a mixed-effects model using pooled data from both experiments, as well as separately for each experimental trial, to determine overall effects as well as differences between trials. The replicate block by which each microcosm was harvested was used as the random effect. Regressions were also performed using mixed-effect models to test whether ecosystem multifunctionality and individual ecosystem functions could be explained by the soil biodiversity index, as well as whether this relationship depended upon the experimental trial (*SI Appendix, Fig. S6*).

In addition to the method of averaging z scores of soil community characteristics, we used partial least-squares path modeling to infer potential direct and indirect effects of soil biodiversity on various ecosystem functions (42). In the path models soil biodiversity was constructed as a latent variable using the measured soil community characteristics as reflective indicators of soil biodiversity. Because all measures of soil biodiversity and ecosystem functions were strongly influenced by the different soil community treatments, we assessed the variation in the measures of soil biodiversity as a direct effect of the variation in all ecosystem functions in the path model. However, the loss of soil biodiversity may have indirectly resulted in changes in some ecosystem functions. Specifically, the effect of the different soil communities on plant productivity and diversity may have consequently indirectly influenced carbon sequestration and nutrient losses through leaching (43, 44). Additionally we also assessed the effects of litter decomposition and N turnover on nutrient losses from the system. Details on all path models presented in *Figs. S9–S12* are given in *SI Appendix, SI Materials and Methods* and in *Tables S2–S5*.

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